# Maple Sirup

# IX. Microorganisms as a Cause of Premature Stoppage of Sap Flow From Maple Tap Holes<sup>1</sup>

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It is a well established fact that undue growth of microorganisms in maple sap adversely affects the color and flavor of sirup produced from it (Edson et al., 1912; Hayward and Pederson, 1946; and Holgate, 1950). It is common experience that maple products produced in the latter part of the season are of lower quality. Recently, plastic bags have been introduced as a substitute for metal or wood buckets for hanging on the tree to collect sap. Sap collected in these bags contains fewer contaminating microorganisms (Naghski and Willits, 1953; and Sproston and Lane, 1953) because these bags are transparent to the sun's sterilizing ultraviolet rays. This could not, however, overcome the deleterious effect of metabolic products in the sap produced by microorganisms growing within the tap hole. In continuing this research, attention was directed to the determination of the extent that microbial development in the tap holes affects the quality of maple products. During these studies, it was observed that infection of tap holes was adventitious, but once the microorganisms attained a foothold and temperatures became favorable, growth proceeded rapidly and could be correlated with premature stoppage of sap flow. It had been previously considered that this stoppage was caused by air and wind getting into the hole and drying out the sapwood.

#### EXPERIMENTAL METHODS

Ordinary tapping. Sugar maple trees were tapped with sterile 7/6-inch bits after first sterilizing the bark with alcohol and flaming. The hole was bored to a depth of 21/2 inches, a sterile spile was driven in, and the collecting container attached. Beyond this, the tap holes received no other special treatment.

Sterile tapping. To determine the characteristics of sap flow under sterile conditions, the following aseptic tapping and collecting methods were used, which are similar to those reported by Holgate (1950). Bits, 7/16 inches in diameter, were wrapped in paper and steri-

lized in the autoclave. The spout apparatus consisted of a rubber stopper for a 5-gallon bottle, fitted with an inverted glass U-tube plugged with cotton to equalize air pressure, and a piece of straight glass tubing for connecting to a spile (closed type) by a short length of rubber tubing. This assembly was also wrapped in paper and sterilized in the autoclave. The bottle was plugged with cotton, covered with a paper cap, and sterilized in a similar manner.

In attaching the apparatus to the tree, a smooth section of the trunk was selected and a thin layer of outer bark was removed with a wood chisel for about 4 square inches around the site selected. The area was then saturated with alcohol and ignited. While this was still burning, a 7/16-inch hole was drilled with the sterile bit and the spile was inserted as rapidly as possible. The tree end of the spile was poked through the paper and seated securely in the tap hole by striking a screw driver held against the shoulder of the spile. The cotton plug was then removed from the bottle and the rest of the paper was removed from the spile assembly, whereupon the attached rubber stopper was quickly inserted in the bottle. Sterile technique and precautions were used throughout, and in this manner it was possible to collect essentially sterile sap (less than 1 microorganism per ml) during the entire season. The completed assembly is shown in figure 1.

Measurements of sap flow. The sap produced by holes tapped and maintained in the normal practice was collected each day on which a flow occurred and the volume was measured to the nearest ounce.

The sap produced by holes tapped and maintained in a sterile condition was collected only when the 5-gallon receiver was nearly filled. The volume of each run was estimated to the nearest quart by calibration on the bottle.

Sampling and culturing of sap. The contents of each of the 5-gallon bottles were thoroughly mixed and a 5- to 10-ml sample of the sap was removed aseptically. Periodically samples of sap were obtained from the open tap holes, by collecting 2 to 3 ml in sterile vials as it dropped from the spile.

Bacterial numbers were determined by culturing appropriate dilutions of sap on nutrient agar (Edson

<sup>&</sup>lt;sup>1</sup> Presented at the 126th meeting of the American Chemical ociety, New York, New York, September 12-17, 1954.

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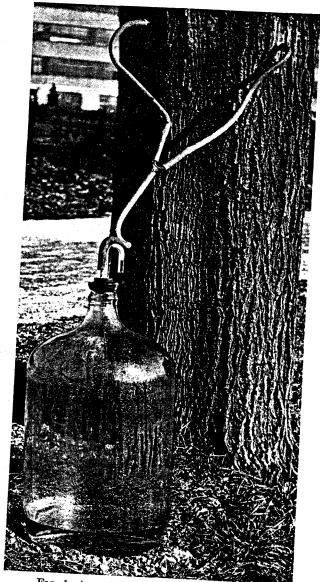


Fig. 1. Assembly for collecting sterile sap

et al., 1912). The presence of yeasts and molds was determined by use of an acidified yeast extract-peptone agar proposed by Wickerham (1951).

### RESULTS

The relationship of bacterial growth in tap holes to premature stoppage (drying up) of sap flow is shown in figure 2. The trees were tapped on January 19, 1954, and three tap holes (A, B, and C) were followed throughout the season. The daily sap flow from these tap holes was almost identical. Samples of sap taken on February 4 were found to be sterile; however, on February 11 that from tap holes A and B showed small numbers of bacteria. The bacterial content of sap from A increased rapidly and on February 18 was 1,700,000 per ml. This was also the last day that any sap flowed from it. Premature drying occurred less than  $4\frac{1}{2}$  weeks after tapping, but only 1 week following contamination.

The bacterial content of sap from hole B increased more gradually and on March 8 showed a count 880,000 per ml. The flow at this time was slow and ceased completely 2 days later; premature drying had occurred 7 weeks after tapping.

In contrast, tap hole C remained sterile for almost 7 weeks even though it had the same exposure to the elements. Bacteria first appeared on March 11, increased progressively, and reached 800,000 per ml on March 24. Two days later, sap flow ceased, 9½ weeks after tapping.

The yield of sap from a sterile tap hole (control) with flow rate similar to that of the three tap holes (A, B, C), is also plotted in figure 2 for comparison. It will be noted that even after tap hole C had become dry there were still three sap flows during which the sterile tap hole produced an additional 26 quarts of sap. On the basis of the yield of sap by the sterile tap hole, premature stoppage reduced the amount of sap produced by tap holes A, B, and C to only 34, 27 and 83 per cent, respectively, of the season's crop.

To eliminate the possible effect of wind or air as a factor in drying of tap holes, four sterile holes were inoculated with a bacterial culture isolated from a

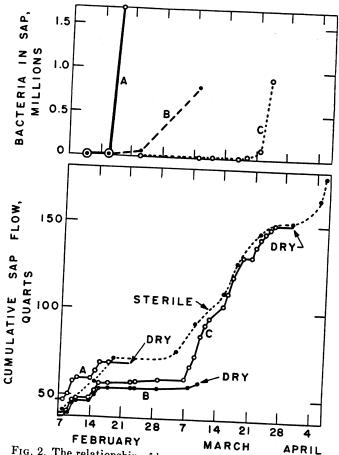


Fig. 2. The relationship of bacterial growth in tap holes premature stoppage of sap flow. All tap holes were made on January 19. Arrow at end of sap flow curve indicates that the tap holes had become dry on this date.

naturally infected tap hole and protected by the enclosed system used in sterile tapping. The results obtained with these holes were compared with four other tap holes which were not enclosed (open spiles) and which became infected naturally. The pattern of bacterial growth and stoppage of sap flow was similar under the two conditions and did not appear to be influenced either by location of the tap hole or date of tapping. Although bacteria were the most frequent cause of premature stoppage of sap flow, several tap holes became dry when molds or yeasts were the predominating microorganisms.

#### DISCUSSION

It has long been recognized by maple sirup producers that early season sap produces the highest quality sirup with the lightest color and delicate maple flavor. However, most producers are reluctant to tap early because it is the common experience that most early tap holes "dry up" prematurely with a resultant loss of a portion of the crop. On the other hand, producers also fail to get several of the earliest and often the best-paying runs because they tap too late. In a recent report Robbins (1953) showed that in 1953, failure to tap in time to catch the sap runs occurring February 20 to 27 resulted in a loss of a third of the crop. Many producers failed to tap in time in spite of the fact that sap flow weather was predicted and announced by the U. S. Weather Bureau for this period.

It is the consensus of opinion (Barraclough, 1952; Bryan et al., 1937; and Collingwood and Cope, 1938) that "drying" of the tap holes is due to air or wind getting into the hole. To counteract this "drying" effect, numerous types of sap spiles have been designed and marketed, but none has proved effective. It had also been observed that "drying" usually followed periods when sap flow was interrupted by abnormally warm weather. Such weather conditions are, of course, conducive to the rapid growth of microorganisms. The weather from February 16 to March 8, 1954 was typical of such periods, with a recorded minimum of 34 F on three days and a maximum of 70 F, and average daily temperatures from 41 to 60 F. The data in figure 2 show that during this 20-day period, sap flows were extremely small and two of the three tap holes went dry. Although tap hole A became dry at the beginning of this period and hole B at the end, under field conditions because of the smallness of the sap flows, the dates of these stoppages would not have been detected until the next normal sap flow occurred.

Besides causing drying of the tap hole, the large numbers of microorganisms that have been observed in infected holes undoubtedly can produce sufficient fermentation products to affect adversely the quality of the sap. Preliminary studies (Naghski, 1953) have shown that sap in which certain bacteria have grown sufficiently to give counts of several million per ml will produce a sirup that is greatly darkened in color. A practical means for controlling tap hole infection in the sugar maples would result in lengthening the season, in increased production of the highest quality early season sirups, and in greatly improved quality of maple sirups produced later in the season.

Further studies on the cause and control of tap hole infection are being initiated at Michigan State College.

# ACKNOWLEDGMENT

The authors thank R. A. Bell for taking temperature readings and making sap flow measurements.

## SUMMARY

Premature "drying" of maple tap holes was correlated with growth of microorganisms and not with physical drying.

Premature stoppage of sap flow from tap holes can reduce the annual yield of sap by 20 per cent to 75 per cent.

The deleterious effects produced by microorganisms growing in the tap holes on the quality of maple products are discussed.

#### REFERENCES

Barraclough, K. E. 1952 Maple Syrup and Sugar Production in New Hampshire. University of New Hampshire Extension Bull. 103.

Bryan, A. H., Hubbard, W. F., and Sherwood, S. F. 1937 Production of Maple Sirup and Sugar. U. S. Dept. Agrl. Farmers' Bull. 1366.

Collingwood, G. H., and Cope, J. A. 1938 Maple Sugar and Sirup. Cornell Extension Bull. 397.

Edson, H. A., Jones, C. H., and Carpenter, C. W. 1912 Micro-organisms of Maple Sap. Vermont Agrl. Expt. Sta. Bull. 167.

HAYWARD, F. W., AND PEDERSON, C. S. 1946 Some Factors Causing Dark-Colored Maple Sirup. New York State Agrl. Expt. Sta. Bull. 718.

HOLGATE, K. C. 1950 Changes in the Composition of Maple Sap During the Tapping Season. New York State Agrl. Expt. Sta. Bull. 742.

NAGHSKI, J., AND WILLITS, C. O. 1953 Maple Sirup. VI. The sterilizing effect of sunlight on maple sap collected in a transparent plastic bag. Food Technol., 7, 81-83.

NAGHSKI, J. 1953 The Organisms of Maple Sap: Their Effect and Control. Report of Proceedings, Second Conference on Maple Products, Nov. 16-18, 1953, p. 48-52. Eastern Regional Research Laboratory, Philadelphia, Pa.

ROBBINS, P. W. 1953 Factors Influencing the Production of High Quality Maple Sap. A progress report. Report of Proceedings, Second Conference on Maple Products, Nov. 16-18, 1953, pp. 34-36. Eastern Regional Research Laboratory, Philadelphia, Pa.

SPROSTON, T. Jr., AND LANE, SUSAN. 1953 Maple Sap Contamination and Maple Sap Buckets. Vermont Agrl. Expt. Sta., Pamphlet 28.

WICKERHAM, L. J. 1951 Taxonomy of Yeasts. U. S. Dept. Agrl. Tech. Bull. 1029.